Diuretic uptitration with half dose combined ACEI + ARB better decrease proteinuria than combined ACEI + ARB uptitration

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Abstract

Background. Residual proteinuria is a strong modifiable risk factor for renal failure progression. We previously showed that the antiproteinuric effect of combined half doses of angiotensin-converting enzyme inhibitor (ACEI) and angiotensin receptor blocker (ARB) is increased by raising diuretic dosage.

Methods. We tested whether uptitration of loop diuretics on top of combined half doses of ACEI and ARB would better decrease proteinuria than uptitration to combined full doses of ACEI and ARB in a randomized, crossover, three periods of 6-week controlled study. Eighteen patients with stable proteinuria over 1 g/day with combined ramipril at 5 mg/day and valsartan at 80 mg/day in addition to conventional antihypertensive treatments were randomized to receive combined ramipril at 5 mg/day and valsartan at 80 mg/day, or combined ramipril at 10 mg/day and valsartan at 160 mg/day, or combined ramipril at 5 mg/day, valsartan at 80 mg/day and increased furosemide dosage in random order. The primary end point was the mean urinary protein/creatinine ratio in two 24-hour urine collections at the end of the three treatment periods. Secondary end points included mean 24-hour proteinuria, home systolic blood pressure (SBP), diastolic blood pressure (DBP), mean arterial blood pressure (MAP) and estimated glomerular filtration rate (eGFR) levels.

Results. The geometric mean urinary protein/creatinine ratio was lower with combined ramipril at 5 mg/day and valsartan at 80 mg/day or combined ramipril at 10 mg/day and valsartan at 160 mg/day, or combined ramipril at 5 mg/day, valsartan at 80 mg/day and increased furosemide dosage compared to combined ramipril at 5 mg/day and valsartan at 80 mg/day, but also to combined ramipril at 10 mg/day and valsartan at 160 mg/day. These differences remained significant after adjustment for SBP, DBP or MAP and 24-hour natriuresis but not after adjustment on eGFR. Diuretic dosage uptitration did not increase the number of home systolic blood pressure measurements below 100 mmHg, but led to a statistically significant increase in the number of symptomatic hypotension episodes.

Conclusions. A cautious uptitration of loop diuretic dosage in addition to combined half doses of ACEI and ARB better decrease proteinuria in patients with CKD and high residual proteinuria than uptitration to full dose of combined ACEI and ARB. This antiproteinuric effect of diuretics was partly explained by an eGFR decrease, suggesting the contribution of haemodynamic modifications, whose safety on the long term still need to be addressed.

Keywords: angiotensin-converting enzyme inhibitor; angiotensin receptor blocker; chronic kidney disease; diuretic; proteinuria

Introduction

Treatment of both high blood pressure and proteinuria over 0.5 g/day is required to control the progression of chronic kidney disease (CKD) [1,2]. Inhibition of the renin–angiotensin system (RAS), through either angiotensin-converting enzyme inhibitors (ACEI) or angiotensin II receptor blockers (ARB), helps to decrease proteinuria and slow down glomerular filtration rate (GFR) decline from the early [3,4] to late stage of CKD [5]. However, despite treatment with ACEI or ARB, many patients present high residual proteinuria and progress to end-stage renal disease (ESRD). Residual proteinuria levels correlate with the rate of GFR decrease, both in diabetic [6] and non-diabetic nephropathies [7,8] and is considered as a strong surrogate end point for CKD progression [9]. Proteinuria might also be a modifiable cardiovascular risk marker in CKD patients [10] and general populations [11]. Therefore, therapeutic strategies should be developed to further decrease proteinuria.

ACEI and ARB antagonize the RAS at different levels, suggesting that their combination may be beneficial. A review reported 14 studies that tested RAS dual blockade by ACEI and ARB versus ACEI or ARB alone [12], and concluded that proteinuria was reduced by the combination compared with ACEI and ARB monotherapies, but this effect was independent of blood pressure only in a minority of these studies. Of note, 10 additional studies had already been published by the time of submission of this later re-
view [13–22]. However, only one study has shown that combined half doses of ACEI and ARB decreased proteinuria better than optimal doses of ACEI or ARB, suggesting a true synergistic antiproteinuric activity [19]. A more comprehensive meta-analysis has now confirmed that combination of ACEI and ARB further reduced proteinuria more than either agent alone, but proved neither a blood pressure independent nor a true synergistic effect.

Controversies arise after serious implausibilities were reported about a single-centre trial [23] that had claimed the superiority of dual RAS blockade on ACEI or ARB monotherapies to prevent doubling of serum creatinine or end-stage renal failure [24]. Furthermore, a recent large controlled study (ongoing telmisartan alone and in combination with ramipril global endpoint trial, ONTARGET) compared the effect of full-dose dual RAS blockade to an ACEI monotherapy on major cardiovascular [25] and renal outcomes [26] in patients with vascular disease or high-risk diabetes, with a mean urinary albumin/creatinine ratio at baseline of 7.2 μg/g in the range of normoalbuminuria, and showed more frequent negative renal events with dual RAS blockade compared to the ACEI monotherapy. However, this deleterious effect of full-dose dual RAS blockade was not observed in the small subgroup of patients with microalbuminuria or overt proteinuria, and the effect of intensive RAS blockade remains debated in patients with high residual proteinuria [26].

The antiproteinuric effect of ACEI may be blunted by high salt intake and can be subsequently restored by combined diuretics. We previously reported that the antiproteinuric effect of combined half doses of ACEI and ARB is increased by raising diuretic dosage [22]. It remains to be determined whether up titration of diuretics on top of combined half doses of ACEI and ARB better decrease proteinuria than up titration to a full dose of combined ACEI and ARB without changing diuretic dosage.

Subjects and methods

Patients population

The inclusion criteria were as follows: age over 18 years; glomerulopathies not requiring immunosuppressive treatments; proteinuria >1 g/24 hours after at least a 2-month run-in period with combined ramipril at 5 mg/day and valsartan at 80 mg/day in addition to conventional antihypertensive treatments; and changes in daily proteinuria <50% at three consecutive tests over a 2-month period. Conventional antihypertensive treatments were unchanged and included calcium channel blockers, beta-blockers, alpha-blockers and centrally active drugs as well as loop diuretics (furosemide at 20 to 120 mg/day) whenever required for a blood pressure goal <130/80 mmHg. Diuretics were also prescribed to prevent pitting oedema. The only patients excluded were those with serum creatinine levels >250 μmol/L (2.82 mg/dL); a serum creatinine level increase >20% following introduction of RAS blocking agents; a contraindication or intolerance to ACEI or ARB; or an office systolic blood pressure (SBP) <110 mmHg or >140 mmHg. Written informed consent was obtained from each patient before inclusion. The protocol was approved by the local ethics committee and conducted according to a Good Clinical Practice guidelines.

Study design

This was a multicentre, prospective, open-label crossover study with concealed centralized randomization with equilibrated blocks of six patients. Patients were assigned to receive in random order either: (a) combined ramipril at 5 mg/day and valsartan at 80 mg/day, (b) combined ramipril at 10 mg/day and valsartan at 160 mg/day or (c) combined ramipril at 5 mg/day, valsartan at 80 mg/day and add-on or increased furosemide dosage. During Period c of the study, furosemide treatment at 40 mg/day was initiated in patients not receiving diuretic therapy at time of inclusion in the study, and furosemide dosage was increased by 40 mg/day in the remaining patients. The furosemide dose could be increased by only 20 mg/day in patients with low systolic blood pressure and high blood urea nitrogen over serum creatinine ratio to avoid pre-renal failure. Patients were advised not to change their usual protein and sodium intake throughout the study.

A Youden square design assured that every treatment was represented in every period with the same frequency to control for period effect (i.e., a time-dependent trend that can affect the experiment as a whole, regardless of the treatment under evaluation). Three treatment sequences were defined (ace, bca and cba), with a treatment factor at three levels and a period factor at three levels. Treatment periods lasted 6 weeks, since the acute haemodynamic effects of a given dose of ACEIs and ARBs on proteinuria are fully reversible within 4 weeks [27]. It was felt unethical to perform a washout with no RAS blocking agents between each treatment period in these patients with severe hypertension and proteinuria.

At the end of each treatment period, two 24-hour urine samples were obtained for protein, creatinine and sodium measurements, and blood was drawn to measure serum creatinine, sodium, potassium and albumin. GFR was estimated (eGFR) using the simplified Modification of Diet in Renal Disease (MDRD) equation [28]. Home systolic and diastolic blood pressure (DBP) were measured and tape printed using a validated apparatus (OMERON 705CP) twice with a 2-minute interval, in the morning as well as in the evening, 3 days per week, during run-in and test–treatment periods. The definition of mean arterial pressure (MAP) was as follows: (SBP 2 × DBP)/3. At the end of each treatment period, a physical examination was performed which included measurement of body weight, heart rate and sitting blood pressure. Symptomatic hypotension and side effects were also searched at this time.

Study measures

The primary end point was the mean urinary protein/creatinine ratio in two consecutive 24-hour collections of urine at the end of each treatment period. Secondary end points were mean 24-hour proteinuria, home SBP, DBP or MAP and eGFR levels. Tolerance was evaluated by the number of home systolic blood pressure measurements below 100 mmHg and the number of symptomatic hypotension episodes.

Sample size

In a previous study, 18 patients were required to detect a clinically as well as statistically significant (P < 0.05) difference in proteinuria levels between four treatment strategies with an 80% power [22]. To compare three treatments in this study, we hypothesized that 18 patients would again be sufficient.

Statistical analysis

The absence of order and carryover effects on the primary efficacy measure (i.e. urinary protein/creatinine ratio) was first confirmed. Analyses were performed on the intention-to-treat population. Linear models (mixed and fixed) were performed. Home SBP, DBP or MAP; eGFR and treatments were considered as fixed effects, and subjects as a random effect. The variables without normal distribution were log transformed for the linear model analysis and reported as geometric mean ± SD.

A primary comparison was made between the three study groups with crude analysis and after adjustment using nested models with the following variables: home SBP, DBP or MAP, 24-hour natriuresis and eGFR. Secondary crude and adjusted comparisons were made between groups to localize the difference using Tukey tests to account for multiple comparisons. Secondary variables, including home blood pressure and eGFR levels, were also compared in crude as well as adjusted analysis.

Tolerance was evaluated by monitoring of kalaemia, number of home SBP measurements below 100 mmHg and cases of symptomatic hypotension using a linear model (mixed and fixed). The level of statistical significance was set to 5% in bilateral situation. The SAS software version 9.1 (SAS Institute, Cary, NC, USA) was used to perform the analyses.
Results

Patients' characteristics at inclusion after run-in with combined ramipril at 5 mg/day and valsartan at 80 mg/day

There were 16 males and two females, all of whom were Caucasians. Their mean age was 53 ± 21 years (Table 1). There were three type 2 diabetics, two immunoglobulin A (IgA) nephropathies, five focal segmental glomerulosclerosis (FSGS) (four secondary and one primary FSGS resistant to corticosteroid treatment), two primary membranous glomerulopathies, one minimal change disease, one mesangio-proliferative glomerulonephritis and four undetermined chronic glomerulopathies. Mean home SBP was 130.7 ± 20.5 mmHg (Table 1). Geometric mean urinary protein/creatinine ratio was 1.47 ×/÷ 1.80 g/g, geometric mean 24-hour proteinuria was 1.97 ×/÷ 1.80 g/day and geometric mean urinary protein/creatinine ratio between combined ramipril at 5 mg/day and valsartan at 80 mg/day (P = 0.6) (Figure 1B and Table 2).

The home mean SBP, DBP or MAP remained unchanged during the study, except in one patient, whose dose of alpha-blockers had to be decreased during the full-dose dual RAS blockade and the increased dosage of furosemide periods, to avoid excessive blood pressure decrease.

Efficacy measurements

There was no statistically significant order (P = 0.28) nor carryover effect (P = 0.17), no patient was lost to follow-up, and there was at least one urinary collection at the end of each treatment period for efficacy evaluation. Salt intakes evaluated by 24-hour urinary sodium remained stable throughout the three treatment periods (Table 2).

The geometric mean of urinary protein/creatinine ratio was significantly lower with combined ramipril at 5 mg/day, valsartan at 80 mg/day and increased dosage of furosemide (33.4 ×/÷ 18.4 mL/min/1.73 m²) compared to combined ramipril at 5 mg/day and valsartan at 80 mg/day (P = 0.007). There was no statistically significant difference in geometric mean eGFR between combined ramipril at 10 mg/day and valsartan at 160 mg/day and valsartan at 80 mg/day (P = 0.3) (Figure 2B and Table 2).

Data are expressed as mean ± SD for value with normal distribution, geometric mean ×/÷ SD for log-transformed variables.
nine ratio was not significantly different between combined ramipril at 10 mg/day and valsartan at 80 mg/day, and combined ramipril at 5 mg/day and valsartan at 80 mg/day ($P = 0.6$). After further adjustment on eGFR, the difference in geometric mean urinary protein/creatinine ratio between combined ramipril at 5 mg/day, valsartan at 80 mg/day and increased dosage of furosemide and combined ramipril at 5 mg/day and valsartan at 80 mg/day was attenuated ($P = 0.10$). Finally, increase of eGFR explained 5% of the decrease of urinary protein/creatinine ratio difference between combined ramipril at 5 mg/day, valsartan at 80 mg/day and increased dosage of furosemide and combined ramipril at 5 mg/day and valsartan at 80 mg/day. Similar results were found with 24-hour proteinuria (data not shown).

**Adverse events**

There were no statistically significant differences in body weight, number of home systolic blood pressure below 100

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### Table 2. Clinical characteristics at the end of each treatment period

<table>
<thead>
<tr>
<th></th>
<th>R5 + V80</th>
<th>R10 + V160</th>
<th>R5 + V80 + F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight (kg)</td>
<td>73.7 ± 17.0</td>
<td>73.0 ± 17.0</td>
<td>72.9 ± 17.2</td>
</tr>
<tr>
<td>Urinary protein/creatinine ratio (g/g)</td>
<td>1.47 ± 1.80</td>
<td>1.30 ± 2.0</td>
<td>0.96 ± 1.95</td>
</tr>
<tr>
<td>24-hour proteinuria (g/day)</td>
<td>1.95 ± 1.80</td>
<td>1.75 ± 1.84</td>
<td>1.20 ± 1.91</td>
</tr>
<tr>
<td>Home SBP (mmHg)</td>
<td>129.5 ± 16.1</td>
<td>125.9 ± 14.1</td>
<td>123.9 ± 18.0</td>
</tr>
<tr>
<td>Home DBP (mmHg)</td>
<td>74.5 ± 7.6</td>
<td>73.1 ± 7.6</td>
<td>70.5 ± 6.4</td>
</tr>
<tr>
<td>Home MAP (mmHg)</td>
<td>92.9 ± 8.3</td>
<td>90.7 ± 7.6</td>
<td>88.3 ± 7.9</td>
</tr>
<tr>
<td>Serum creatinine (μmol/L)</td>
<td>164 ± 15</td>
<td>172 ± 15</td>
<td>190 ± 17</td>
</tr>
<tr>
<td>eGFR (mL/min/1.73 m²)</td>
<td>40.4 ± 16.6</td>
<td>38.1 ± 16.8</td>
<td>33.4 ± 18.4</td>
</tr>
<tr>
<td>Creatinine clearance (ml/min)</td>
<td>72 ± 1.68</td>
<td>68 ± 1.63</td>
<td>59 ± 1.8</td>
</tr>
<tr>
<td>24-hour urinary sodium (mmol/day)</td>
<td>4.6 ± 0.6</td>
<td>4.6 ± 0.6</td>
<td>4.6 ± 0.6</td>
</tr>
<tr>
<td>No. of home SBP &lt;100mmHg</td>
<td>1.2 ± 2.5</td>
<td>2.2 ± 4.3</td>
<td>2.9 ± 4.0</td>
</tr>
<tr>
<td>No. of symptomatic hypotension</td>
<td>0 ± 0</td>
<td>0.05 ± 0.2</td>
<td>1.0 ± 2.6</td>
</tr>
</tbody>
</table>

R5 + V80: combined ramipril at 5 mg/day and valsartan at 80 mg/day; R10 + V160: combined ramipril at 10 mg/day and valsartan at 160 mg/day; R5 + V80 + F: combined ramipril at 5 mg/day, valsartan at 80 mg/day and increased furosemide dosage. Data are expressed as mean ± SD or geometric mean ×/÷ SD.

*a*Comparison R5 + V80 versus R5 + V80 + F; *P* < 0.05.

*b*Comparison R10 + V160 versus R5 + V80 + F; *P* < 0.05.

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**Fig. 1.** Urinary protein/creatinine ratio (g/g) (**A**), proteinuria levels (g/day) (**B**), in patients with combined ramipril at 5 mg/day and valsartan at 80 mg/day (R5 + V80), combined ramipril at 10 mg/day and valsartan at 160 mg/day (R10 + V160) and combined ramipril at 5 mg/day, valsartan at 80 mg/day and increased furosemide dosage (R5 + V80 + F).
mmHg and serum potassium levels between treatments (Table 2). However, there were significantly more symptomatic hypotensive episodes with combined ramipril at 5 mg/day, valsartan at 80 mg/day and increased dosage of furosemide compared to combined ramipril at 5 mg/day and valsartan at 80 mg/day (P < 0.05). The number of symptomatic hypotensions was not significantly different between combined ramipril at 10 mg/day and valsartan at 160 mg/day, and combined ramipril at 5 mg/day and valsartan at 80 mg/day (P = 0.10).

Discussion

The residual proteinuria level is a strong surrogate end point for CKD progression [9], and combination of ACEI and ARB further reduced proteinuria more than either agent alone [29]. We previously showed that an increase of diuretic dosage on top of combined half doses of ACEI and ARB better decrease high residual proteinuria than maximum recommended doses of ACEI and ARB monotherapies, or combined half doses of ACEI and ARB without changing the diuretic dosage [22]. We here confirm that, to decrease high residual proteinuria, the next step to combined half doses of ACEI and ARB could be an introduction or a modest uptitration of diuretics rather than uptitration to full doses of combined ACEI and ARB.

Combining a low-sodium diet (below 6 g/day NaCl) to unchanged doses of diuretics is also an effective method to maximize the antiproteinuric efficacy of RAS blockade [30]. Indeed, high salt intake decreases both the antihypertensive and the antiproteinuric effect of RAS blocking agents [31]. The harmful effect of high salt intake is further emphasized by an epidemiological study in a general population, since levels of 24-hour natriuresis and blood pressure correlated independently with proteinuria [32]. Inversely, the benefit of salt restriction may be overlooked, since in very low protein diet, urinary urea excretion directly correlated with urinary sodium excretion, and therefore, the decrease of salt intake may contribute to the renoprotective effect of dietary protein restriction [33]. Twenty-four-hour urinary sodium excretion was similar during the three treatment periods. Indeed, patients were recommended to keep their diet constant throughout the study. We did not measure urinary urea excretion. However, since there is a strong correlation between sodium and protein intake [33], a stable sodium intake suggests that protein intake was not significantly modified during the course of our study.

In our study, the antiproteinuric effect of a modest diuretic dosage introduction or uptitration was observed in patients with moderate sodium intakes (i.e. geometric mean of 162 mmol/day, i.e. 9.5 g/day NaCl), and despite no statistically significant decrease in body weight. Nested models were performed to evaluate the treatment effect with multivariate adjustment. After adjustment on blood pressure, the treatment effect decreased but remained significant. However, in our study, a significant decrease in eGFR after diuretic uptitration explained part of the observed proteinuria decrease, suggesting a contribution of haemodynamic modifications. This eGFR decrease was in the range of the target changes for long-term kidney pro-
Diuretic and RAS blockade

are now required. Thirdly, this aggressive RAS blocking agents, and long-term comparative studies on incident ESRD progression, our study is a short-term trial on surrogate criteria, and long-term comparative studies on incident ESRD are now required. Thirdly, this aggressive RAS blocking agents on renal failure progression.

Similarly, part of the beneficial effect of aldosterone blockers may be secondary to their natriuretic effect and the control of an overnourished overhydration. Indeed, two placebo-controlled studies with a crossover design claimed that spironolactone can decrease urinary protein excretion [35,36]. However, in these two studies, spironolactone also decreased systolic blood pressure (6–10 mmHg) and body weight (1.2–1.4 kg) after an 8-week treatment period, demonstrating a significant natriuretic effect [35,36]. The effect of aldosterone blockade was compared with various antihypertensive drugs [37], including RAS blocking agents [38–41]. All but one study [38] showed the superiority of the aldosterone blockade strategy for proteinuria decrease. However, these observations matched our data with loop diuretic reinforcement [22]. Finally, a 1-year placebo-controlled study with a parallel groups design suggested that spironolactone add-on to ACEI and/or ARB may further decrease proteinuria after two months [42]. However, no controlled study compared aldosterone blockade to increased dosage of loop diuretics on top of RAS blocking agents. Therefore, it remains uncertain that the effect of aldosterone blockade on proteinuria is independent of its diuretic property.

Uptitration to full doses of combined ramipril and valsartan did not statistically decrease the urinary protein/creatinine ratio compared to combined half doses of RAS blocking agents. In contrast, increase dosage of ARB over their maximum recommended dose may also help to control proteinuria. Indeed, forced titration up to ultrahigh doses of monotherapies with candesartan [43], irbesartan [44], valsartan [45] and telmisartan [46] can further decrease proteinuria, but these strategies have not been compared to combined ACEI and ARB, and the tolerability of such strategies would also need to be examined.

Our study has several limitations. Firstly, there was no washout between the three treatments. However, the evaluation was made at the end of a 6-week treatment period, and the acute haemodynamic effects of a given dose of ACEI and ARB on proteinuria are fully reversible within 4 weeks [27], and even faster with loop diuretics. This was further supported by the absence of statistically significant order and carryover effects in this study. Secondly, although residual proteinuria is a strong predictor of CKD progression, our study is a short-term trial on surrogate criteria, and long-term comparative studies on incident ESRD are now required. Thirdly, this aggressive RAS blocking strategy with dual blockade combined to diuretic uptitration may expose to pre-renal failure. In our study, body weight was not significantly decreased after a cautious diuretic uptitration, suggesting mainly intra-renal haemodynamic modifications. However, in the ONTARGET study, enhancing RAS blockade with full dose combined ACEI and ARB was associated with a significant increase of acute reversible renal failure episodes after acute diarrhoea or fever [26]. We can anticipate that our strategy of diuretic uptitration may also increase the risk of reversible pre-renal failure after acute dehydration. Indeed, a close monitoring of pre-renal failure is mandatory in patients with combined RAS blocking agents and loop diuretics. Furthermore, symptomatic hypotension episodes need to be monitored carefully, particularly in elderly patients.

We conclude that a cautious uptitration of loop diuretic dosage in addition to combined half doses of ACEI and ARB better decrease proteinuria in patients with CKD and high residual proteinuria than uptitration to full dose of combined ACEI and ARB. Therefore, even aggressive RAS blocking strategies may be blunted by sodium retention. This antiproteinuric effect of diuretics was partly explained by an eGFR decrease, suggesting the contribution of haemodynamic modifications, whose safety on the long term still need to be addressed.

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Conflicts of interest statement. None declared.

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